MOVEMENT OF CYCLOHEXIMIDE IN RICE PLANTS

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The possibility of using cycloheximide (Actidione) against brown spot (*Helminthosporium oryzae* var. *Breda*) of rice plants (*Oryza sativa* Linn. var. *kele*) by foliar spray has been explored. It has been observed that an early spray of minimum inhibitory concentration of cycloheximide (100 μg/ml) reduces the amount of infection on the host by about 80–90 per cent and thereby controls the fungus. At the same concentration of the antibiotic a belated spray on the 25th day after inoculation could reduce the infection by only 10–15 per cent. An overdose of the antibiotic at this stage of infection has not been attempted. Local inhibition of *Helminthosporium oryzae* infection of rice plants extends the possibility of inhibiting the obligate parasite growing on the host by a physiological systemic control. It is observed that cycloheximide is absorbed and translocated from the sprayed leaves both in the upward and the downward direction. By the method of bio-assay the antibiotic could be identified in the roots and the young leaves of healthy plants immediately following antibiotic spray. But, possibly due to decomposition of cycloheximide in aqueous cell sap, the systemic control of infection could not be attained.

INTRODUCTION

Most of our knowledge on the movement of antibiotics in higher plants has been reviewed by Crowdy and Pramer (1955*a*), later by Pramer (1959) and Goodman (1959). There is evidence that several antibiotics are absorbed and translocated in higher plants, prominent among them being penicillin, chloromycetin, streptomycin, griseofulvin, etc. (Crowdy and Pramer 1955*a*; Crowdy *et al.* 1956), whereas subtilin, neomycin, etc., fall into a group that is neither absorbed nor translocated after injections (Pramer 1959). It is generally believed that the neutral and acidic antibiotics (penicillin, chloromycetin) move more readily in plants than the basic (neomycin, streptomycin) or amphoteric (tetracyclines) group. Streptomycins and tetracyclines move more freely in some plants than in others (Pramer 1954; Gray 1958). The movement of cycloheximide in rice plants has been studied in this laboratory, and it forms the subject of the present paper. In view of the ability of antibiotics to be translocated in higher plants (Brian 1957; Crowdy

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and Pramer 1955*b*), it is possible that disease may be partially controlled by some form of systemic protection.

In the present investigation the penetration of cycloheximide into the leaves of rice plants and its translocation thence to other parts of the plant, namely to the upper new leaves and to the roots, were studied with special reference to brown spot of rice caused by *Helminthosporium oryzae*.

Materials and Methods

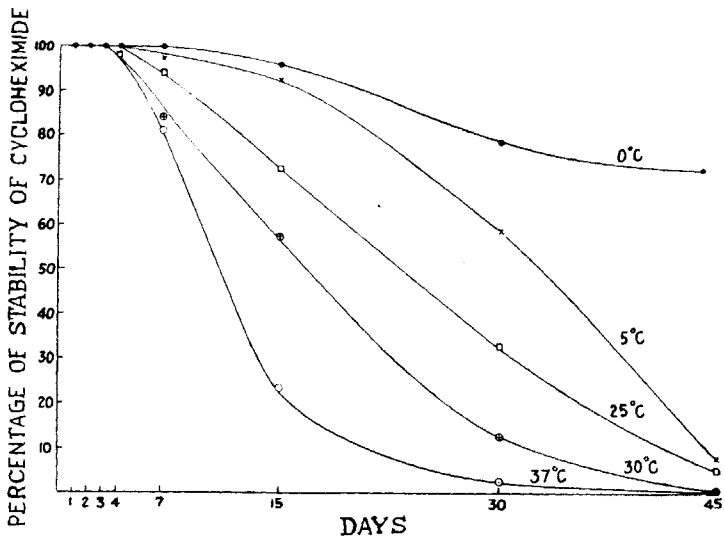
The host.—*Oryza sativa* L. var. *kele* (Chinsura) was used as the host plant. This particular *aus* variety is a high land crop and is suitable for pot experiments.

The pathogen.—*Helminthosporium oryzae* var. *Breda* was obtained from the State Agricultural College, West Bengal, which was found to be one of the pathogenic races of *Helminthosporium oryzae* infecting the *kele* variety of rice. For spraying, a spore suspension of $1{\cdot}6 \times 10^6$ was prepared in 5 per cent glycerine to increase stickiness when adhered to the host. The suspension was preserved at 5° C as the standard inoculum. Two sprays, one early in the morning and the other in the evening, were given.

The antibiotic.—Cycloheximide, marketed as Actidione, was obtained through the kind courtesy of Upjohn Co., Kalamazoo, Michigan, U.S.A. The dose of minimum inhibitory concentration (100 μg/ml), as observed in *in vitro* studies, was used for spraying the leaves. It was observed by hanging drop culture method that at 100 μg/ml concentration of the antibiotic spore germination of *H. oryzae* was completely inhibited. For each treatment two light sprays, one early in the morning and another late in the afternoon, were given. A third spray of the same potency (100 μg/ml) of the antibiotic proved to be toxic to the host, when sprayed within 48 hours of the first treatment. Since no attempt had been made to control the temperature during the course of the experiment, it was found necessary to study the stability of the antibiotic in aqueous phase in a wide range of temperature. It would be evident from Text-fig. 1 that during the first four days the decomposition of the antibiotic was practically nil, whereafter a slow decomposition above 25° C was noted. From the 15th day onward a rapid decomposition took place between 25° C and 37° C. To keep a uniform potency of the antibiotic, fresh solution was made for every treatment.

Experimental.—The experiment was designed to study (1) penetration of cycloheximide into leaves, (2) translocation of cycloheximide from sprayed leaves, and (3) systemic activity of the antibiotic. The experiment was conducted on potted plants for a period of 60 days. Seeds were sown by broadcasting in March in well-manured alluvial soil in pots. Three days after sowing a preliminary selection of rice seedlings was made leaving about 20 well-germinated seedlings in each pot. A final selection of ten rice plants

of almost equal height and foliage per pot was made after ten days. Besides, two other plants were marked out for intermediate observations on translocation. The pots were kept in a well-aerated shady spot to hasten infection and lessen the time of drying of the sprays. The inoculum was first sprayed on the 15th day after sowing and repeated the next day. Each plant was at the 3- or 4-leaved stage. Uniformity regarding age of the crop, inoculum and antibiotic was maintained as far as practicable.



TEXT-FIG. 1. Stability of cycloheximide in aqueous solution at different temperatures.

In all five treatments were laid out. Besides the control, four treatments of antibiotic were given on different days. They were, after inoculation, in order of 1st, 3rd, 5th and 25th days. Only two sprays of antibiotic per treatment, at the concentration of 100 μg/ml, were given. For studying the systemic activity of the antibiotic a fresh spray of inoculum was made simultaneously on all plants after the completion of the experimental period of 60 days.

During the course of the experiment the pots were kept flooded with water. The counts of lesions of *Helminthosporium* infections were made at the end of every seven days.

RESULTS AND DISCUSSION

I. *Penetration of cycloheximide into leaves*

The immediate effect of cycloheximide in reducing the severity of *Helminthosporium oryzae* infections on rice plants would be evident from Table I.

TABLE I

Effects of foliar spray of cycloheximide on infected rice plants previously inoculated with Helminthosporium oryzae. *The treatments T_1, T_2, T_3, T_4 correspond to plants sprayed with cycloheximide on the 1st, 3rd, 5th and 25th day after inoculation*

Plant No.	Control			T_1			T_2			T_3			T_4		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	9	117·2	12	8	108·9	2	7	96·2	2	9	115·4	4	10	126·4	9
2	11	142·6	8	9	112·6	1	8	112·1	2	8	103·8	5	8	109·8	7
3	9	121·5	14	12	152·2	2	5	81·3	2	6	82·7	3	10	119·5	6
4	8	107·3	6	9	118·4	2	7	91·4	3	6	78·5	4	9	119·6	7
5	10	114·8	7	10	108·0	1	9	114·6	4	9	119·2	5	6	79·8	6
6	12	158·0	9	7	92·3	—	6	87·6	3	7	101·6	4	9	122·1	7
7	10	134·4	8	9	104·7	—	6	85·2	—	8	107·0	7	10	132·6	9
8	8	98·7	10	8	106·9	—	8	109·3	—	6	75·4	3	7	81·8	8
9	9	132·6	13	10	115·1	1	9	116·8	—	7	81·9	3	8	103·7	5
10	8	110·9	9	5	120·3	—	7	96·6	—	9	113·1	2	7	94·1	2
Average	9·4	12·3	9·6	8·7	11·3	0·9	7·2	9·9	1·6	7·5	9·7	4	8·4	10·9	6·6

A—number of leaves.
B—total area of leaves (cm^2).
C—number of lesions.

The infection, in terms of the number of lesions per sq. cm area of leaf surface, was reduced by more than 60 per cent when antibiotic was sprayed on the 1st, 3rd or 5th day of inoculation. The antibiotic spray on the 25th day could decrease the infection by only about 10 per cent, but by this time most of the leaves were damaged through infection spots. Translocation of the antibiotic could hardly have taken place through the damaged wood-elements, and the possibility of a systemic control of disease was also greatly reduced. Almost equal number of lesions were counted on the younger leaves and on the older leaves in this case, whereas in the first three treatments the lesions on the newly-formed leaves were far less in number and, in some cases, completely absent. There is every reason to assume that translocation of antibiotic took place before the latter underwent decomposition within the host.

Analysis of covariance was applied to eliminate the influence of area on the number of lesions, but the reduction of experimental error in comparison with the error obtained by analysis of variance was not appreciable. So, simple analysis of variance was used on the number of lesions per plant (Table II).

By statistical analysis, significant variation in treatments was observed. Actidione had a distinct effect in reducing the number of lesions on leaves. The earlier the application, the greater was the beneficial effect. Though

TABLE II

Analysis of variance in terms of lesions per treatment

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Ratio (F)
Treatment	4	520·32	130·08	29·23 *
Error	45	200·10	4·45	
Total	49	720·42		

S.E. $= \sqrt{0{\cdot}4450} = 0{\cdot}67$

* Indicates significance beyond 1 per cent level.

there existed no significant difference between treatments T_1 and T_2, they were significantly different from treatments T_3 and T_4.

II. *Translocation of cycloheximide from sprayed leaves*

Translocation of cycloheximide to different regions of the host plant was determined by plating tissues on 2 per cent malt-agar seeded with *Helminthosporium oryzae*. No chemical evidence for the presence of antibiotic was sought. It may be mentioned in this connection that Crowdy *et al.* (1955, 1956) found quantitative estimates of antibiotic in the tissues by bioassay to agree well with chemical determinations. The presence of cycloheximide was noted on various parts of the host, namely lamellae of new leaves, leaf bases, stem bases and roots, up to the 7th day after treatment. But no antibiotic could be identified in those regions on the assay plates 45 days after treatment. The temperature during the period varied between 23° C and 33° C. Since no attempt had been made to artificially control the environmental temperature, it is presumed that a normal decomposition of cycloheximide in aqueous phase (Text-fig. 1) might have resulted in its absence within the tissues of the host at the completion of the experimental period.

III. *Systemic activity of cycloheximide*

It may seem presumptive to speak of systemic control of plant disease, when the antibiotic itself was undergoing a breakdown within the tissues of the host. But a close study of the reduction of infection by cycloheximide spray showed, apart from its direct effect, that a partial systemic control by translocation into growing regions was effected for a limited period.

The second inoculation with *H. oryzae*, by spraying of all the plants at the end of the experimental period, resulted in equal amount of infection on all the four sets of plants treated with antibiotic. It is reasonable to conclude that cycloheximide is ineffective as a systemic fungicide against brown spot of rice.

Since foliar infections could be controlled by the antibiotic to a certain extent, it is possible that the latter subscribes more towards local inhibitory activity in the control of leaf infection.

Analogous experiments by Gray (1955, 1958) with streptomycin and noformicin, and by Wallen and Millar (1957) with cycloheximide, yielded evidence of translocation of antibiotics in both directions. But no clear evidence of systemic activity could be obtained. Dye (1956) found no direct evidence of truly systemic activity of streptomycin in peach leaves.

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